## **Original Articles**

## Reproductive Biology Section

# Successful pregnancies from embryos cryopreserved more than ten years: two case reports

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#### Summary

Purpose: To determine the efficacy of using embryos cryopreserved more than ten years in a donor embryo program.

Methods: Embryos were cryopreserved using a simplified freezing protocol and then donated for anonymous use after the donor had had a successful pregnancy and was sure she did not want to conceive again.

Results: Two women in the donor embryo program transferred embryos that had been cryopreserved longer than ten years. Both patients delivered healthy babies.

Conclusions: Embryos cryopreserved over ten years can result in successful pregnancies.

Key words: Frozen transfer, Longevity, Live delivery.

#### Introduction

Currently many patients who are waiting to receive donor embryos have expressed concern over the amount of time an embryo can be cryopreserved and still remain viable. Some of this concern is the result of the time limit many countries allow embryos to remain cryopreserved. To date, the longest cryopreserved embryo used by a donor embryo recipient achieving a successful pregnancy was just over nine years [1]. The cases presented here had two full-term deliveries from embryos cryopreserved for 10.8 and 11.8 years.

#### Case Report

Case Report n. 1

A 35-year-old woman and her male partner required the use of frozen donated embryos related to premature ovarian failure and a severe male factor problem. The embryos the couple chose had been cryopreserved in January 1993 for a total time of 10.8 years in frozen storage. The embryos were cryopreserved using a simplified freezing protocol with one-step removal of the cryoprotectant 1,2 propanediol [2]. A total of four embryos were available for thawing. Three were frozen at the 4-cell stage and one was frozen at the 7-cell stage. Three of the embryos survived the thaw. Two embryos were transferred after 72 hours. Assisted embryo hatching was performed prior to transfer [3]. One was a 9-cell and the other was an 8-cell at the time of transfer. Both embryos had good morphology. The age of the donor at the time of retrieval was 27.9 years. The patient conceived and delivered a healthy full term boy.

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#### Case Report n. 2

A 31-year-old single woman required the use of frozen donated embryos due to premature ovarian failure and no male partner. The embryos the woman chose had been cryopreserved in October 1992 for a total time of 11.8 years in frozen storage. A total of six embryos were available for thawing. Three were frozen at the 2 pronuclear stage, two were frozen at the 4-cell stage, and one was frozen at the six-cell stage. All of the embryos survived the thaw. Three embryos were transferred after 72 hours. All three embryos were 8-cell at the time of transfer. All embryos had good morphology. Two of the embryos were of sufficient quality to be refrozen [4]. The age of the donor at the time of retrieval was 38.0 years. The patient conceived twins and delivered a healthy, full-term boy and girl. To date, the refrozen embryos have not been chosen by any other patients involved in the donor embryo program.

#### Discussion

The frozen donor embryo program allows either single women or women with partners to become pregnant without the major expense incurred with regular IVF or with the use of a donor egg. Also for religious reasons some individuals cannot have contact with another person's gametes. The amount of time an embryo can be cryopreserved and still remain viable has been of great concern to both frozen embryo recipients and also to frozen embryo donors. Some of the reasoning behind this concern is the length of time other countries allow embryos to be cryopreserved, as well as the limited pregnancy information available on embryos frozen for long periods of time.

Although there are no studies showing a decrease in embryo viability based on the length of time embryos have been cryopreserved, there are countries which legislate the amount of time an embryo can remain frozen. In Western Australia, legislation states that embryos can only remain cryopreserved for a length of three years, unless a special judicial request is made by the legal guardians of the embryos [5]. The United Kingdom also has laws governing the length of time an embryo may remain in storage. Embryos stored in the U.K. may remain frozen for five years, which may be extended to a maximum of ten years [6].

The frozen donor embryo program allows patients with multiple infertility factors to forego the expense incurred with a regular IVF cycle or with the use of a donor egg. The main drawback patients have with a donor embryo program is the often unknown quality of embryos being donated to the program. Although the expense is minimal compared to other programs, frozen donor embryo recipients are still hesitant to chose embryos that have been stored for long periods of time since little information is available concerning their possible viability. Patients are also hesitant to chose embryos of uncertain viability due to the length of time most patients have to wait prior to receiving frozen donor embryos. Since the average waiting time for donor embryos is approximately eight months to a year, many patients prefer to wait for embryos that have been cryopreserved a relatively shorter period of time.

Patients wishing to donate embryos are also under the assumption that embryos frozen for long periods of time are not viable. As a result many embryos that could potentially be used to produce pregnancies are now donated for research or simply discarded.

These case reports demonstrate that even embryos cryopreserved for more than ten years are still viable and capable of producing healthy babies. This information may help potential embryo donors make an informed decision concerning the eventual disposition of their embryos. Patients waiting to receive donated frozen embryos may also benefit from this knowledge. They will now be able to choose embryos frozen for long periods of time without having to consider the possible consequences of cryopreservation and time in storage.

We believe that these cases represent the longest on record that embryos have been frozen and successfully resulted in a live pregnancy after thawing and subsequent transfer. The previous longest one was nine years and interestingly was also donated to an anonymous couple [1]. These embryos had been frozen at the 2 pronuclear stage [1]. Thus, case 2 presented here now represents the longest time on record of frozen embryos resulting in a live pregnancy and the longest known time that embryos originally frozen at the multi-cell stage have been cryopreserved before thawing with the embryo transfer resulting in a live delivery.

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